At Siemens Healthineers, our purpose is to enable healthcare providers to increase value by empowering them on their journey towards expanding precision medicine, transforming care delivery, and improving patient experience, all enabled by digitalizing healthcare.

An estimated 5 million patients globally benefit every day from our innovative technologies and services in the areas of diagnostic and therapeutic imaging, laboratory diagnostics and molecular medicine, as well as digital health and enterprise services.

We are a leading medical technology company with over 170 years of experience and 18,000 patents globally. With more than 48,000 dedicated colleagues in 75 countries, we will continue to innovate and shape the future of healthcare.

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References:
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Evaluation of the VERSANT kPCR Sample Prep

Quality Nucleic Acid Extraction from a Wide Variety of Specimens with Nano Bead Technology

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Successfully Automating the Molecular Laboratory

Evaluation of the VERSANT kPCR Sample Prep for Extraction of Quality Nucleic Acids from a Wide Variety of Specimens

| **Siemens Healthineers** 250 nm* | **Competitor A** MagNA Pure LC Total NA Kit 3200 nm* | **Competitor B** m2000 Sample Preparation System DNA 1200 nm* | **Competitor C** QIAsymphony Virus and Bacterial Midi Kit 4500 nm* |

Figure 1. Electron microscopic images of commercially-available magnetic silica beads.

Executive Summary

Molecular diagnostics and life-science studies depend on the ability to effectively extract high-quality nucleic acids. Siemens Healthineers offers an automated, high throughput system that can extract nucleic acids from 1 to 96 samples in a single batch run. The VERSANT® kPCR Sample Prep and VERSANT® Sample Preparation 1.0 Reagents kit (SP 1.0) and Sample Preparation 1.2 Reagents kit (SP 1.2) enable extraction of both DNA and RNA from a wide variety of specimen types.

Siemens Healthineers magnetic nanobead technology enables exceptional RNA and DNA recovery, with sensitive and reproducible target detection in real-time PCR applications. In addition, the system offers the flexibility to combine different specimen types in the same run and the versatility to use one specimen for up to six PCR assays by splitting the eluate. Together, the VERSANT kPCR Sample Prep and IVD-labeled kits provide an excellent solution for extraction of nucleic acid for a variety of molecular applications.
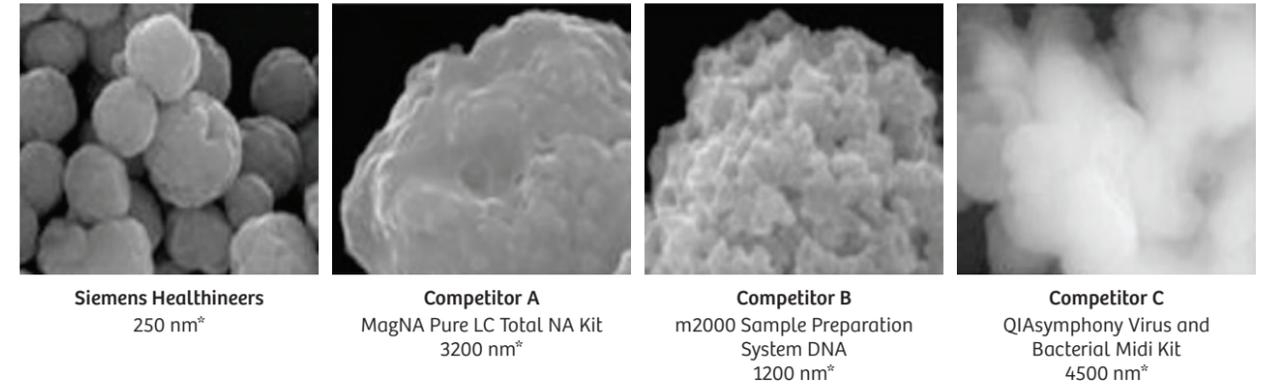
Magnetic Particle-based Isolation Technologies in Molecular Diagnostics

Nucleic acid isolation for use in in vitro diagnostic molecular testing has traditionally been labor-intensive and lacking robust reproducibility and sensitivity. For more than a decade, extraction technologies have employed magnetic particles coated with silica, which allowed the development of an automated extraction process. However, many automated protocols have produced inconsistent results.[1]

Siemens Healthineers has developed a method that applies an entirely different approach to synthesizing silica-containing magnetic particles for nucleic acid isolation. Unlike most other magnetic beads, Siemens Healthineers uses iron oxide rather than silica, along with a photolithographic toner as the base.

These chemically pure iron oxide particles are further modified in a subsequent nanotechnology production step in which an ultrathin layer of silica is deposited onto the surface of the particles. The Siemens Healthineers technology results in very small (<1 um) and homogeneous particles that provide unique advantages such as very efficient washing that results in purer eluates, pipetting reproducibility, rapid magnetization, and improved homogeneous suspension. The smooth surface area of the particles allows high purification yields, so that even small quantities of nucleic acids can be isolated from comparatively large matrix volumes. Figure 1 shows a comparison of commercially-available magnetic silica beads as viewed with electron microscopy.

*Indicated mean diameter evaluated by semiautomatic imaging processing routines from high-resolution scanning electron microscopy.

High Sensitivity for RNA Extraction

Method Comparison

The VERSANT kPCR Sample Prep and SP 1.0 reagents[†] deliver increased RNA extraction recovery and provide better protection from sample interference compared to other commercially-available sample preparation platforms

Figure 2 shows a comparison of the VERSANT kPCR Sample Prep with SP 1.0 reagents versus four other commercially-available methods in the extraction and detection of norovirus RNA from 39 stool samples obtained from norovirus-infected patients.

The four commercially-available automated platforms tested were:

• EasyMAG (bioMerieux)

• m2000sp (Abbott)

• MagNA Pure LC 2.0 (Roche)

• QIAsymphony (Qiagen)

VERSANT kPCR Sample Prep extraction efficiency was compared to the other extraction technologies by using (1) similar sample input and output volumes and (2) an identical real-time PCR cycler (VERSANT kPCR AD[‡] unit) and CE-marked assay for amplification and detection of norovirus RNA (AnDiaTec).RNA was extracted with each extraction technology by using identical stool aliquots for each patient-sample.

Overall, the Siemens Healthineers method provided up to a 7-fold (maximum 2.89 Ct mean difference between VERSANT kPCR Sample Prep and QIAsymphony; Figure 2)[1] more sensitive extraction recovery for norovirus RNA from stool samples than the other methods. In contrast to the other commercial platforms, all samples tested with the VERSANT kPCR Sample Prep and SP 1.0 reagents tested positive for both the target RNA and the spiked RNA-based internal controls.
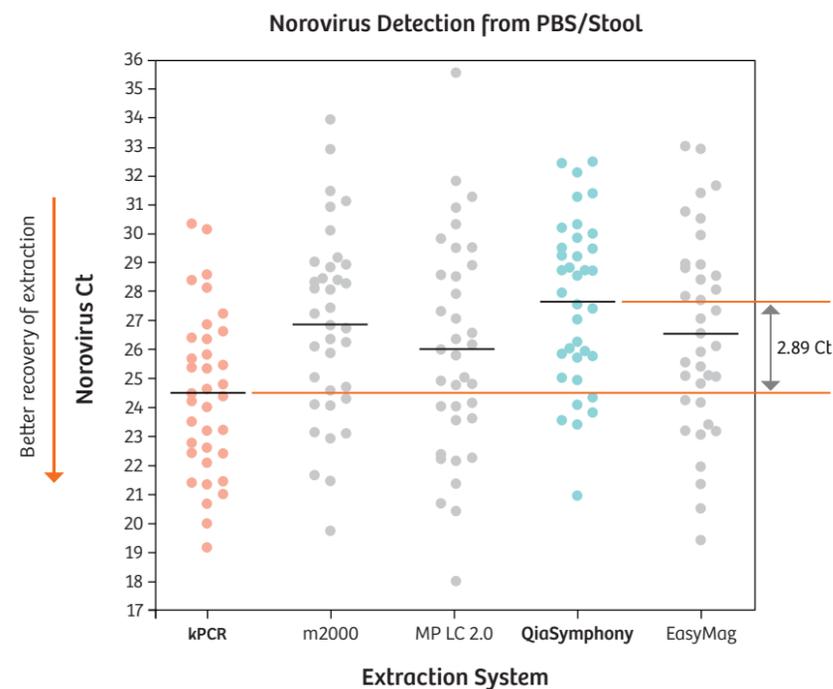

Norovirus Detection from PBS/Stool

Figure 2. Comparison of the VERSANT kPCR Sample Prep with four commercially-available automated extraction platforms.

†CE-marked for IVD use in the EU. Class I in the U.S. Product availability may vary by country.
‡CE-IVD marked for use in the EU. Not available for sale in the U.S. Product availability may vary by country.

High Sensitivity for DNA Extraction

Method Comparison

The VERSANT kPCR Sample Prep and SP 1.2 reagents deliver higher DNA yield compared to other commercially-available preparation platforms.

Five whole-blood specimens from cytomegalovirus (CMV)-infected patients were processed using three different extraction technologies—QIAsymphony (Qiagen), MagNA Pure LC 2.0 (Roche Diagnostics), and VERSANT kPCR Sample Prep—with their respective whole-blood sample preparation reagent kits.

CMV viral load was measured and calibrated to copies/mL by external quantification standards in the DNA eluates obtained from these platforms using the CMV R-gene kit (Argene/bioMerieux) on the VERSANT AD module of the VERSANT kPCR Molecular System[§] from Siemens Healthineers. Sample input and eluate output amounts were equivalent across all three platforms.

Table 1 compares the extraction performance of the three systems after quantification into copies/mL for the same five aliquoted whole-blood patient samples.

The VERSANT kPCR Sample Prep and SP 1.2 reagents consistently produced higher viral load results on the same set of patient samples (Table 1).[2] Higher quantification in copies/mL originated from lower detected Ct values between the three platforms, which correlates with better DNA extraction sensitivity.

Table 1. CMV viral load measurement of patient samples processed using different sample preparation platforms.

Quantitation (copies/mL)			
Clinical Sample Number	MagNA Pure LC 2.0	QIAsymphony	kPCR
1	2467	5219	17,250
2	618	1713	2471
3	372	1094	22,650
4	167	164	1539
5	6666	6601	13,350
P-value** Against kPCR	0.011	0.019	–

§Not available for sale in the U.S.
**P-value of less than 0.05 means that the VERSANT SP 1.2 Reagents kit—along with the VERSANT kPCR Sample Prep— yielded significantly higher quantitations than the other methods. A two-sample paired t-test of log quantitation was used.

Product availability may vary by country.

A Universal Extraction Solution for Diverse Sample Types

Method Comparison

VERSANT Sample Preparation Reagents kits (SP 1.0 and SP 1.2) provide high recovery of DNA and RNA from a wide variety of clinical specimen types using the VERSANT kPCR Sample Prep.

Recovery of nucleic acids from clinical samples was evaluated using the VERSANT kPCR Sample Prep with both the VERSANT Sample Preparation 1.0 Reagents (optimized for total nucleic acid extraction, but also amenable for DNA) and the VERSANT Sample Preparation 1.2 Reagents (optimized for DNA only extraction) kits. Each sample type was spiked with 10 ng of human breast adenocarcinoma total RNA (MCF-7) or human genomic DNA (gDNA). Seven to ten independent samples of each sample type were analyzed. Real-time PCR assays targeting housekeeping genes RPL37A for MCF-7 RNA and PAEP for gDNA were used for amplification and quantification of RNA or DNA.

Recovery was evaluated by comparing the concentration of MCF-7 RNA or gDNA in each sample (minus endogenous nucleic acid) to the same amount of MCF-7 RNA or gDNA amplified without going through the sample extraction process. When necessary, samples were pretreated with lysis buffer or PBS (VERSANT Sample Preparation 1.0 Reagents kit) or pretreatment buffer (VERSANT Sample Preparation 1.2 Reagents kit).

Recovery of bacterial DNA was evaluated using Chlamydia trachomatis (CT) cells (supplied by Advanced Biotechnologies Inc.) spiked into ThinPrep and SurePath culture media (commercially-available collection media for vaginal swabs) at a concentration of 10^6, 10^5, and 10^4 copies/mL run in triplicate. Samples were extracted using both the VERSANT Sample Preparation 1.0 and 1.2 Reagents kits.

Reagents kits, then amplified using the VERSANT CT/CG DNA 1.0 assay (kPCR). To evaluate extraction, recovery of nucleic acids, CT reference standard DNA was amplified without sample extraction and compared to CT cells amplified with extraction at the same starting concentration.

DNA and RNA were successfully purified from all clinical specimen types tested (Table 2).[3] The SP 1.0 Reagents are optimal for extraction of RNA only and combined DNA and RNA from one sample; the SP 1.2 Reagents are optimal for extraction of DNA only.

CT bacterial DNA was recovered in both ThinPrep and SurePath collection media at all levels tested, indicating that bacterial DNA in these sample types can be isolated with both SP 1.0 and SP 1.2 Reagents with similar efficiency (Table 3).[4]

Table 2. DNA/RNA mean recovery in clinical samples.

Sample	Pretreatment Required	SP 1.0 Average RNA Recovery (%)	SP 1.0 Average DNA Recovery (%)	SP 1.2 Average DNA Recovery (%)
Amniotic fluid	Yes	78	29	72
Ascites	Yes	66	36	95
BAL	Yes	66	53	140
Breast milk	Yes	97	36	57
Buccal swab	Yes	66	37	47
Cells in culture media	Yes	65	57	68
CSF	Yes	81	39	71
Eye swab	Yes	64	34	86
NP swabs	Yes	79	40	116
PBMC	Yes	71	85	78
Plasma	No	56	15	70
Semen††	Yes	36	>100	45
Serum	No	45	15	118
Sputum	Yes	57	82	93
Stool	Yes	67	42	60
Urine‡‡	Yes	Not tested	Not tested	132
Vaginal swabs‡‡	Yes	Not tested	Not tested	76
Whole blood§§	Yes	Not tested	Not tested	94

††These samples have high background DNA.
‡‡These sample types were not evaluated with SP 1.0 Reagents in this study, as they had previously been validated for use in a CE-marked assay.
§§For use with VERSANT Sample Preparation 1.2 Reagents only.

Table 3. Bacterial DNA mean recovery in cell transport media.

Culture Medium	CT DNA Spike (copies/mL)	SP 1.0 Reagents (Average Ct)	SP 1.2 Reagents (Average Ct)
ThinPrep	1,000,000	23.1	22.3
	100,000	26.4	25.8
	10,000	29.9	29.0
SurePath	1,000,000	23.5	23.8
	100,000	26.8	26.8
	10,000	30.0	30.0

Flexibility and Workflow Consolidation

The VERSANT kPCR Sample Prep is flexible allowing the extraction of a variable batch size of 1 to 96 samples in about 3 hours. Furthermore, sample types may be mixed in an individual batch run. There are nine open-channel extraction protocols that allow for adjustment of different sample input and eluate output volumes.

The VERSANT kPCR Sample with MiPLX Software provides an innovative approach to improving workflow and consolidation in the molecular laboratory. The combination of the VERSANT kPCR Sample Prep with MiPLX provides the flexibility to not only extract nucleic acids from a variety of specimens but also assay set-up of laboratory-developed and other laboratory's assays.***

VERSANT MiPLX Software introduces dynamic protocols that:

- Automate sample extraction from a wide variety of sample types in one run.
- Automate PCR assay setup for a more efficient workflow.
- Enable assay set-up for laboratory's assays.
- Provide the ability to get more information out of a single sample extraction by splitting the eluate instead of the sample.
- Allow one, two, four, or six assays to be run from one extracted sample on a single PCR plate through multiplexing capabilities.

Summary

The VERSANT kPCR Sample Prep includes features that enhance the quality and recovery of nucleic acids from a wide variety of specimen types while maximizing the laboratory's efficiency and productivity.

- A consolidated platform, with a universal protocol and reagents for the isolation of DNA and RNA from diverse clinical specimens, minimizes equipment needs, inventory requirements, and QC management.
- Iron oxide beads (<1 μm in size) coated with a nanolayer of silica improves reproducibility, recovery, and quality, resulting in enhanced assay performance.
- Two IVD-labeled kits provide for the extraction of DNA and RNA from a large variety of sample types.
- The VERSANT kPCR Sample Prep features fast, easy setup and walkaway operation.
- The VERSANT kPCR Sample Prep provides high throughput, with up to 96 samples extracted per run in about 3 hours.
- Extraction of high-quality nucleic acids supports a wide-range of molecular diagnostic applications, including sequencing, end-point PCR, hybridization assays, and real-time PCR.
- Nine extraction protocols provide flexibility for sample input and eluate output volumes.
- Dynamic protocols allow automated real-time PCR assay setup, including analysis of up to six assays from one sample eluate in one run.

***The laboratory is responsible for verification and validation of the assay.